Cross Resistance to Insecticides in Malathion- and Fenitrothion-resistant Strains of the Smaller Brown Planthopper, Laodelphax striatellus Fallén

Cross Resistance to Insecticides in Malathion- and Fenitrothion-resistant Strains of the Smaller Brown Planthopper, *Laodelphax striatellus* Fallén. Kozaburo OZAKI and Tatsuo KASSAI (Division of Phytopathology and Entomology, Kagawa Agricultural Experiment Station, Takamatsu, Kagawa, Japan) Received April 21, 1971. *Botyu-Kagaku* 36, 111, (1971)

16. ヒメトビウンカのマラソンおよびフェニトロチオン抵抗性系統の殺虫剤に対する交差抵抗性

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ヒメトビウンカのマラソンとフェニトロチオン抵抗性系統の殺虫剤に対する交差抵抗性を明らかにするため，両抵抗性系統の有機りん系，環状リン酸エステル系，カーバメイト系と有機塩素系の各種殺虫剤に対する LD_{50} 値を感受性系統のそれらに比較した。マラソンに87倍の抵抗性を示すマラソン抵抗性 (Rm) 系統はダイアジノン，メチルパラオキソン，フェニトロオキソン，カヤホス，シディアル，EPN とサリオキソンに抵抗性であり，またパラチオン，メチルパラチオン，フェニトロチオン，フェンチオン，メカルバム，マラオキソンとサリチオンに対しても抵抗性が少し増大した。フェニトロチオンに23倍の抵抗性を示すフェニトロチオン抵抗性 (Rf) 系統はマラソンに45倍，シディアルに27倍といった高い抵抗性をもたらすとともに，パラチオン，メチルパラチオン，フェンチオン，ダイアジノン，メチルパラオキソン，フェニトロオキソン，カヤホス，マラオキソン，EPN とサリチオンに抵抗性を示した。またこの系統は DDVP，CVP，ダイシストン，メカルバムとバミドエイトに対する抵抗性も少し増大した。Rm と Rf 系統は，感受性系統におけると同様，カーバメイト系の各種殺虫剤に感受性であった。DDT と γ-BHC に対して，Rf 系統の LD_{50} は感受性系統のそれらと同等であったが，Rm 系統の LD_{50} は感受性系統におけるより低かった。

Introduction

A strain of the smaller brown planthopper, *Laodelphax striatellus* Fallén, rapidly developed the resistance to malathion and fenitrothion through successive selections. Many individuals of these selected strains showed a high activity of E_7 band in electrophoretic zymogram of esterases which hydrolyze beta-naphtyl acetate[10]. It was suggested that the development of resistance of the said planthopper to malathion and fenitrothion was closely correlated to the activity of the esterase of E_7 band accordingly. It was further suggested that both strains selected for malathion and fenitrothion might have a common basis mechanism of resistance. If so, it may be deduced that the cross-resistance pattern would be similar with these malathion- and fenitrothion-resistant strains.

The resistance pattern of the field population of a given insect pest species is generally the reflection of the kind and amount of insecticides previously used in the field. The resistance of the smaller brown planthopper to malathion and other organophosphorus insecticides is recently posing a serious problem in the chemical control of this insect because of the development of cross-resistance to various insecticides. The present study was therefore undertaken in order to evaluate the toxicity of various insecticides to a susceptible strain and two strains resistant to malathion or fenitrothion respectively with the aim to investigate the extent of cross-resistance pattern of resistant strains.

Materials and Methods

Fifth instar larvae of respective strains were used in all experiments. The history of the susceptible LE strain was described previously[11] and the resistant Rm and Rf strains were obtained from the said strain through a continuous selection with either malathion or fenitrothion, giving 70 to 75 percent mortality to respective generations by contact method. Selections were conducted for 17 generations and survived individuals were reared for further two to three generations without any insecticidal pressure in order

Table 1. Name and purity of active ingredient of insecticides tested.

Name	Purity	Name	Purity
Parathion	97.5	Fujithion (*O,O*-dimethyl *S*-*p*-chlorophenyl phosphorothioate)	90.0
Methyl parathion	98.4		
Fenitrothion	99.6		
Fenthion	97.1	EPN	93.4
Diazinon	94.1	CYP (*O*-*p*-cyanophenyl *O*-ethyl phenylphosphonothioate)	89.9
CYAP (*O*-*p*-cyanophenyl *O,O*-dimethyl phosphorothioate)	96.0	Salithion (2-methoxy-4H-1,3,2-benzodioxaphosphorin-2-sulfide)	Pure
Paraoxon	Pure		
Methyl paraoxon	Pure	Salioxon (2-methoxy-4H-1,3,2-benzodioxaphosphorin-2-oxide)	Pure
Fenitro-oxon	Pure		
DDVP	98.0	Carbaryl	Pure
CVP (2-chloro-1-(2,4-dichlorophenyl) vinyl diethyl phosphate)	94.2	CPMC (2-chlorophenyl *N*-methylcarbamate)	Pure
Kayaphos (4-methylthiophenyl dipropyl phosphate)	Pure	MTMC (3-methylphenyl *N*-methylcarbamate)	Pure
		MPMC (3,4-dimethylphenyl *N*-methylcarbamate)	Pure
Disulfoton	Pure	BPMC (2-sec-butylphenyl *N*-methylcarbamate)	Pure
Dimethoate	97.0		
Malathion	Pure	PHC (2-isopropoxyphenyl *N*-methylcarbamate)	98.0
Cidial	95.5		
Mecarbam	85.0	APC (4-diallylamino-3,5-dimethylphenyl *N*-methylcarbamate)	Pure
Malaoxon	Pure		
Vamidoate	Pure	DDT and γ-BHC	Pure

to secure enough number of individuals for determining the level of resistance to various insecticides. All planthoppers were reared on rice seedlings in a rearing cage under the controlled condition of sixteen hour illumination and at 25 ±1°C, renewing rice seedlings weekly.

Insecticidal compounds tested were enumerated in Table 1, and the dosage mortality data for those compounds were obtained by a contact method proceeded as follows. Two microliter of acetone solution of different concentrations of insecticide was dropped the inside of a glass tube, 1.1 cm in diameter and 10.4 cm in length, by a micrometer cylinge. Fifty microliter of acetone was added to each tube in order to distribute the insecticide uniformly on the inner surface of the tube. After the solvent evaporated completely, ten fifth instar larvae of the smaller brown planthopper were placed into each tube. Larvae were kept in contact with insecticidal film for three hours, then they were transferred into a glass tube, 2 cm in diameter and 10.5 cm in length, containing the rice seedlings, and were kept at 25°C. Mortality counts were made after 21 hours.

Results

LD_{50} values for 33 different insecticides including organophosphates, saligenin cyclic phosphates, carbamates and chlorinated hydrocarbons of LE, Rm and Rf strains were shown in Table 2. It was indicated that methyl parathion, fenitrothion, CYAP, methyl paraoxon, Salithion, MTMC and PHC were highly toxic to the LE strain, while CVP, dimethoate, mecarbam, Vamidoate, EPN, CYP, APC, DDT and gamma-BHC were less toxic than formerly mentioned insecticides. Rm strain was found as susceptible as LE strain to DDVP, CVP, disulfoton, dimethoate, Vamido-

Table 2. Toxicity of various insecticides to the susceptible (LE), malathion-(Rm) and fenitrothion-resistant (Rf) strains of smaller brown plant-hopper.

Insecticide	LE strain		Rm strain		Rf strain	
	1/b	LD_{50}*	1/b	LD_{50}	1/b	LD_{50}
Parathion	0.20	0.127	0.30	0.318	0.54	0.766
M-parathion	0.19	0.026	0.33	0.080	0.22	0.225
Fenitrothion	0.15	0.040	0.41	0.151	0.57	0.921
Fenthion	0.24	0.286	0.39	0.989	0.35	3.42
Diazinon	0.20	0.143	0.27	0.771	0.38	0.783
CYAP	0.18	0.040	—	—	—	—
Paraoxon	0.32	0.168	—	—	—	—
M-paraoxon	0.18	0.033	0.30	0.295	0.47	0.519
Fenitro-oxon	0.30	0.084	0.51	0.509	0.42	1.52
DDVP	0.24	0.081	0.52	0.147	0.19	0.252
CVP	0.34	1.21	0.30	1.55	0.28	3.10
Kayaphos	0.26	0.297	0.30	1.48	0.29	1.71
Disulfoton	0.24	0.078	0.37	0.152	0.26	0.305
Dimethoate	0.26	0.944	0.34	1.08	0.28	1.31
Malathion	0.26	0.242	0.58	20.99	0.43	10.84
Cidial	0.32	0.204	0.45	1.96	0.52	5.51
Mecarbam	0.18	0.906	0.32	2.43	0.29	2.25
Malaoxon	0.26	0.203	0.31	0.658	0.39	1.99
Vamidoate	0.21	1.25	0.43	2.45	0.74	3.95
Fujithion	0.30	0.299	0.44	0.506	0.27	0.496
EPN	0.29	0.553	0.49	9.10	0.47	4.98
CYP	0.20	1.20	—	—	—	—
Salithion	0.18	0.024	0.32	0.084	0.40	0.134
Salioxon	0.13	0.062	0.24	0.426	—	—
Carbaryl	0.20	0.379	0.28	0.299	0.30	0.311
CPMC	0.27	0.052	0.21	0.052	0.19	0.055
MTMC	0.13	0.029	0.22	0.050	0.13	0.052
MPMC	0.32	0.102	0.37	0.101	0.24	0.084
BPMC	0.30	0.085	0.41	0.083	0.30	0.163
PHC	0.24	0.049	0.31	0.049	0.31	0.066
APC	0.28	0.855	0.33	1.32	0.25	1.70
DDT	0.66	7.18	0.38	1.89	0.68	7.18
γ-CHC	0.60	0.538	0.50	0.210	0.60	0.507

* LD_{50} in μg per tube (1.1×10.4cm)

ate, Fujithion and carbamates of carbaryl, CPMC, MTMC, MPMC, BPMC, PHC and APC. Rf strain was almost as susceptible as LE strain to dimethoate, Fujithion, six carbamates mentioned previously, DDT and gamma-BHC. It should be noted that LD_{50} for DDT and gamma-BHC of Rm strain were somewhat smaller as compared with competent values of LE strain.

LD_{50} values for most organophosphates and saligenin cyclic phosphates of Rm and Rf strains were higher than the comparative values of LE strain. Levels of resistance as expressed by the ratio of LD_{50} of the resistant strains to that of the susceptible strain to respective insecticides were given in Table 3.

Table 3 shows that Rm strain having 87-fold resistance to malathion revealed the cross-resistance of the magnitude of 17-fold to EPN and from 5- to 10-fold to diazinon, methyl paraoxon, fenitro-oxon, Kayaphos, Cidial and Salioxon. A slight increase in the resistance up to two- to four-fold was observed with parathion, methyl parathion, fenitrothion, fenthion, mecarbam, malaoxon and Salithion. Rf strain having a 23-fold resistance to fenitrothion as compared with LE strain revealed a high level of resistance to malathion (45-fold) and Cidial (27-fold). The said strain showed a cross-resistance to fenthion, methyl paraoxon and fenitro-oxon in the magnitude of more than 10-fold and to parathion, methyl parathion, diazinon, Kayaphos, malaoxon, EPN and Salithion in the magnitude of 5- to 10-fold. The said strain showed only a slight resistance of two- to four-fold to DDVP, CVP, disulfoton, mecarbam and Vamidoate.

Table 3. Resistance spectra of malathion-(Rm) and fenitrothion-resistant (Rf) strains of smaller brown planthopper.

Insecticide	Resistance level (Ratio at LD_{50})	
	Rm strain	Rf strain
Parathion	2.5	6.0
M-parathion	3.1	8.7
Fenitrothion	3.8	23.0
Fenthion	3.5	12.0
Diazinon	5.4	5.5
M-paraoxon	8.9	15.7
Fenitro-oxon	6.1	18.1
DDVP	1.8	3.1
CVP	1.3	2.6
Kayaphos	5.0	5.7
Disulfoton	1.9	3.9
Dimethoate	1.1	1.4
Malathion	86.7	44.8
Cidial	9.6	27.0
Mecarbam	2.7	2.5
Malaoxon	3.2	9.8
Vamidoate	2.0	3.2
Fujithion	1.7	1.7
EPN	16.5	9.0
Salithion	3.5	5.6
Salioxon	6.9	—
Carbaryl	−1.3	−1.2
CPMC	1.0	1.1
MTMC	1.7	1.8
MPMC	1.0	−1.2
BPMC	1.0	1.9
PHC	1.0	1.3
APC	1.5	2.0
DDT	−3.8	1.0
γ-BHC	−2.6	−1.1

Discussion

Busvine *et al.* (1963)[3] reported that malathion resistant colony of *Chrysomya putoria* showed a very specific resistance to malathion and malaoxon and that this character in *Chrysomya putoria* was in accordance with the results of previous studies on malathion-resistant strains of *Musca domestica* and *Culex tarsalis*. However, it was found in this study that the Rm strain of the smaller brown planthopper resistant to malathion showed cross-resistance to certain other organophosphates. Tanaka *et al.* (1967)[12] formerly demonstrated that malathion-resistant strain of the green rice leafhopper, *Nephotettix cincticeps*, which was 98-fold as resistant as normal strain, was highly cross-resistant to dimethoate (137-fold), Cidial (37-fold) and parathion (17-fold), and moderately resistant to mecarbam, EPN, fenitrothion, diazinon and fenthion (more than 5-fold). The results obtained in this study with malathion-resistant strain of the smaller brown planthopper was rather similar with the finding made by Tanaka *et al.* (1967)[12] with the green rice leafhopper with an exception that the planthopper was not cross-resistant to dimethoate.

Generally speaking, it can be said that the level of resistance of a given insect pest goes up when a particular compound has been used repeatedly for a extended period against the said insect pest. Forgash and Hansens (1959)[5] found that diazinon resistant strain (38-fold) of the housefly, *Musca domestica*, was resistant to chlorthion (23-fold), parathion (16-fold) and ronnel (18-fold) and moderately resistant to malathion (5-fold). Busvine (1959)[2] found that field strains of the housefly distributing in Italy and Denmark were resistant to diazinon and parathion but not to malathion. The Rf strain of the smaller brown planthopper showed the highest level of resistant to malathion and was demonstrated to have a wide resistance spectrum to organophosphorus insecticides in this study. The resistance pattern of Rf strain of the smaller brown planthopper was considered as foundamentally different from that of OP-resistant strains of the housefly.

It was already reported that the strains of housefly resistant to organophosphates such as diazinon, malathion, chlorthion and parathion were cross-resistant to carbamates as well[4,5,6,7,9]. Kimura and Nakazawa (1968)[8] reported the existence of low degree of cross-resistance to carbaryl and MTMC in the malathion-resistant strain of the smaller brown planthopper. Although Rm and Rf strains of the smaller brown planthopper investigated in this study were demonstrated as not cross-resistant to carbamate insecticides, it is suspected that the smaller brown planthopper resistant to malathion may have to some extent the cross-resistance to carbamate insecticides in certain cases.

Brown (1960)[1] summarized some cases in which OP-resistant strains of insect pest showed a extremely high degree of cross-resistance to hydrocarbon insecticides. In the present study no definite cross-resistance to DDT and gamma-BHC was observed with the Rf strain. It is interesting to note further that Rm strain showed a slightly higher susceptibility to DDT and gamma-BHC than LE strain. The enhanced susceptibility to DDT and gamma-BHC in Rm strain was in accordance with the results of studies made on malathion resistant green rice leafhopper and smaller brown planthopper[8,12]. It is implicated that the development of resistance to malathion may induce a slight negative cross-resistance to DDT and gamma-BHC.

It was observed from the table indicating the level of resistance that both Rm and Rf strains were highly resistant to malathion, that they have developed more resistance to malathion than to malaoxon, and to methyl paraoxon than to methyl parathion, that they have developed similar level of resistance each other to diazinon, Kayaphos and mecarbam, but to dimethoate, Fujithion and six carbamate insecticides. It was also observed that while Rf strain showed low degree of resistance to DDVP, CVP, disulfoton and Vamidoate, Rm strain failed to develop resistance to these insecticides, Rm strain could increase the resistance only slightly to those insecticides to which Rf strain could develop a relatively high resistance. It was furthermore observed that both the Rm and Rf strains have developed resistance to phosphate and thiophosphate compounds with cyclic radicals such as phenyl and pyrimidinyl and to compounds with carboxy ester linkage. These findings may imply that the mechanism of resistance existing in Rm and Rf strains is not substantially different and that two common major factors concerning the splitting of *O*-phenyl linkage and hydrolyzing carboxy ester are existing in Rm and Rf strains. Since Rf strain was slightly more resistant than Rm strain to many of organophosphorus insecticides tested in this study, additional factor or factors concerning OP-resistance may be involved in the Rf strain.

Summary

LD_{50} values for 33 insecticides including organophosphates, saligenin cyclic phosphates, carbamates and chlorinated hydrocarbons of malathion-and fenitrothion-resistant (Rm and Rf) strains of the smaller brown planthopper were compared with those values of susceptible (LE) strain by contact method with the aim to clarify the cross-resistance of the resistant strains to various insecticides. Rm strain which showed 87-fold resistance to malathion was resistant to diazinon, methyl paraoxon, fenitro-oxon, Kayaphos, Cidial, EPN and Salioxon. A slight increase in the resistance to parathion, methyl parathion, fenitrothion, fenthion, mecarbam, malaoxon and Salithion was also observed with this strain. Rf strain which showed 23-fold resistance to fenitrothion was found as resistant to parathion, methyl parathion, fenthion, diazinon, methyl paraoxon, fenitro- oxon, Kayaphos, malathion, Cidial, malaoxon,

EPN and Salithion. The said strain showed a slight increase in the resistance to DDVP, CVP, disulfoton, mecarbam and Vamidoate. However, both Rm and Rf strains were found as susceptible to carbamates such as carbaryl, CPMC, MTMC, MPMC, BPMC, PHC and APC to the same degree as with the susceptible LE strain. LD_{50} values of Rf strain for DDT and gamma-BHC were similar with those values obtained with LE strain, but the competent values of Rm strain were rather smaller than those of the susceptible LE strain.

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Metabolic Fate of Proparthrin. Studies on Insecticide. VIII. Michio NAKANISHI, Yasuyuki KATO, Tetsuya FURUTA, Seiji MIURA. (Research Laboratories, Yoshitomi Pharmaceutical Industries, Ltd. Fukuoka-Pref.) Received May 10, 1971. *Botyu-Kagaku*, 36, 116, (1971). (with English Summary 121).

17. プロパルスリンの生体内における消長

殺虫剤に関する研究 第8報[1] 中西美智夫, 加藤安之, 古田哲弥, 三浦誠二 (吉富製薬株式会社 研究所) 46. 5. 10. 受理

新しい菊酸誘導体, 2-methyl-5-(2-propynyl)-3-furylmethyl 2, 2-dimethyl-3-(2-methyl-1-propenyl)-cyclopropane-carboxylate (プロパルスリン) の ^{3}H 標識体 (^{3}H—プロパルスリン) を合成し, ラットにおける吸収, 排泄, 生体内分布および代謝について検討した.

ラットに ^{3}H—プロパルスリン 100mg/kg を経口投与すると, 4日以内の尿および糞中にそれぞれ投与した ^{3}H の40%および35%が, また腹腔内投与ではそれぞれ38%および28%が排泄された. 24時間以内の胆汁中には40%が排泄された. 本化合物またはその放射性代謝産物はほぼ全身的に分布するが, 血液 (血清) 以外の組織中からの消失は速やかであった.

血液中の放射性物質は主として血清中に存在し, 未変化体はほとんど存在しなかった.

ラットの尿および胆汁中に未変化体は検出されず, 主代謝産物はプロパルスリンの加水分解産物である 3-hydroxymethyl-2-methyl-5-(2-propynyl)-furan の glucuronide であった.

プロパルスリンは著者らの研究所で新規合成された菊酸誘導体[2]で, 本化合物の殺虫効果[3]および毒性についてはすでに詳細な検討がなされている.

著者らは本化合物のほ乳動物における生体内運命を解明する目的で ^{3}H 標識化合物 (IV) (以下 ^{3}H—プロパルスリンと略) を合成し, ラットを用い吸収, 排泄, 生体内分布および代謝について検討したので報告する.